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The Felt Fern Genus *Pyrrosia* Mirbel (Polypodiaceae): a New Subgeneric Classification with a Molecular Phylogenic Analysis Based on Three Plastid Markers

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Pyrrosia Mirb. (Polypodiaceae) comprises about 59 species, most of which occur in Southeast Asia, but some taxa are also in Africa and Oceania. Although several authors have investigated the species diversity of the genus, a phylogenetic study focusing on the relationship between species has not been available. We used sequences of three plastid markers (*rbcL*, *rps4-trnS* and *atpB*) to reconstruct a phylogenetic tree for 38 taxa of *Pyrrosia*. Trees were generated under Bayesian inference, ML analysis and MP analysis. Groups present in all analyses were used to propose a subgeneric classification of *Pyrrosia*. As a result, six subgenera, *P.* subgenus *Lunae* Vasques subgenus nov., *P.* subgenus *Neoniphopsis* (Nakai) Vasques comb. & stat. nov., *P.* subgenus *Niphobolus* (Kaulf.) Vasques comb. nov., *P.* subgenus *Niphopsis* (J. Sm.) Shing, *P.* subgenus *Pyrrosia* and *P.* subgenus *Solis* Vasques subg. nov., are proposed and an identification key, diagnostic traits, notes for identification and an overall discussion of each subgenus are given. The phylogenetic tree represents about 65% of the diversity of the genus. The remaining species are assigned to the proposed subgenera based on their diagnostic traits.

Key words: molecular phylogeny, plastid markers, Polypodiaceae, *Pyrrosia*, subgeneric classification

Pyrrosia Mirb. (Polypodiaceae) is a genus of about 59 species of epiphytic and epipetric ferns with simple leaves occurring in tropical and temperate regions of the Old World with the highest diversity in the eastern Himalaya and Sumatra (Hovenkamp 1986, Shing & Iwatsuki 1997). The genus is included in the platycerioid group Polypodiaceae, sharing with its sister group, *Platycerium* Desv., indumentum composed of stellate hairs (Nayar & Chandra 1967, Hoshizaki 1972, Crabbe *et al*. 1975, Hovenkamp 1986). *Pyrrosia* is popularly known as felt ferns due to the presence of stellate hairs, and wool-like hairs in some species, covering the lamina. Simple leaves with stellate hairs are a distinguishing trait for *Pyrrosia*.

Pyrrosia was established in 1806 based on *P. chinensis* Mirb. (= *P. stigmosa* (Sw.) Ching) as the only species (Mirbel 1803). According to Shing (1983), the genus name was long overlooked by pteridologists, being recognized instead under such names as *Cyclophorus* Desv. and *Niphobolus* Kaulf. During the 19th and 20th centuries, several taxonomies for *Pyrrosia* were proposed, with species constantly being relocated to other genera (Desvaux 1811, Kaulfuss 1824, Presl 1836, Smith 1857, Giesenhagen 1901, Underwood 1903, Christensen 1906, 1929, van Alderwerelt van Rosenburgh 1908, Nakai 1928, Farwell 1931, Ching 1933, 1935, Nayar 1961, Nayar & Chandra 1965, 1967, Price 1974, Hovenkamp 1984).

Hovenkamp's (1986) extensive revision of the genus included 57 species arranged in 10 groups

based on morphological, anatomical, ecological and distributional data. Although Hovenkamp (1986) presented a novel classification for the species, as well as distribution maps, descriptions, keys for identification and illustrations. he considered his groups to be informal, since formal recognition would probably result in a series of *species incertae sedis* and many para- or polyphyletic taxa.

Shing & Iwatsuki (1997) also produced an extensive taxonomic treatment that included 64 species. They suggested dividing *Pyrrosia* into two subgenera: subgenus *Pyrrosia* represented by two sections, five series and 62 species; and subgenus *Niphobolus*, represented by two species, while stafing that the species concept in Hovenkamp's work was too wide to allow an understanding of the structure of the lineages native to Asia. Due mainly to the high occurrence of homoplastic characters, Hovenkamp (1986) was unable to infer synapomorphic traits that could sustain the proposed groupings, or even the genus *Pyrrosia* as a whole (Shing & Iwatsuki 1997).

Additional studies (Schneider *et al*. 2004, Kreier & Schneider 2006, Tsutsumi & Kato 2006, Schuettpelz & Pryer 2007, Kim *et al*. 2013) of the evolutionary history of *Pyrrosia* and other closely related genera, mostly focused on the platycerioid ferns (i.e. *Platycerium* and *Pyrrosia*), have placed the genus in the polygrammoid fern group.

Although the close evolutionary relationship with *Platycerium* is well supported, the recognition of infrageneric groups within *Pyrrosia*, as well as the assignment of synapomorphies that may support the monophyly of the genus and of the infrageneric groups, has not been well known. In this context, the main goal of this research was to generate a molecular phylogeny of *Pyrrosia*, including a representative number of species, and to provide a new taxonomic treatment.

Materials and Methods

Sampling and DNA extraction

Our sampling included 38 species of *Pyrrosia*, including DNA samples and fresh leaf samples. Fresh leaves were first dried in silica gel and DNA was extracted using a DNeasy plant mini kit (QIAGEN). All the DNA samples obtained were immediately stocked at −30°C in completely dark boxes. As the outgroup, sequences of two species of *Platycerium* were included in the analysis. Detailed information for the samples, the sequences analyzed and accession numbers for the sequences are in Appendix 1.

Amplification and sequencing

Sequences of three different plastid DNA regions were amplified using the primers cited in Appendix 2. The amplification reactions included: initial denaturation at 94°C for 3 min (*rbcL*) and 95°C for 5 min (*rps4-trnS*, *atpB*); 35 cycles of amplification under 94°C for 45 sec, 57°C for 45 sec, 72°C for 1 min 10 sec (*rbcL*), 95°C for 30 sec, bottom-up grade of 50–55°C for 30 sec, 72°C for 1 min (*rsp4-trnS*), and 95°C for 30 sec, bottom-up grade of 57–62°C for 30 sec, 72°C for 1 min (*atpB*); and final extension at 72°C for 10 min. The PCR products were purified using materials from Wizard PCR Clean-Up system (PROMEGA) and following the standard protocol for this kit. Dye Terminator Cycle sequencing reactions were performed using 2μL of GenomeLab DTCS quick-start kit's premix (Beckman Coulter), 1 μL of the same premix buffer, 1.6 μL of the target primer, 1 μL of PCR sample and 4.4 μL of milliQ water. The thermal cycle conditions for the dye reaction were as follows: 30 cycles of 96°C for 30 seconds, 50°C for 20 seconds and 60°C for 4 minutes, followed by a cooling to 4°C. Finally, the samples were sequenced using a CEQ 8000 sequencer (Beckman Coulter). All the sequences acquired in this research are deposited in GenBank; their accession numbers are presented in Appendix 1.

Alignment and phylogenetic analysis

The sequences were aligned using ClustalX v.2.0.12 (Larkin *et al.* 2007) and by manual revision. Sequences acquired from GenBank (Appendix 1) were also used, and missing data was included in the final alignment (Zheng & Wiens 2015). Using jModelTest v.2.1.7 (Darriba *et al.*

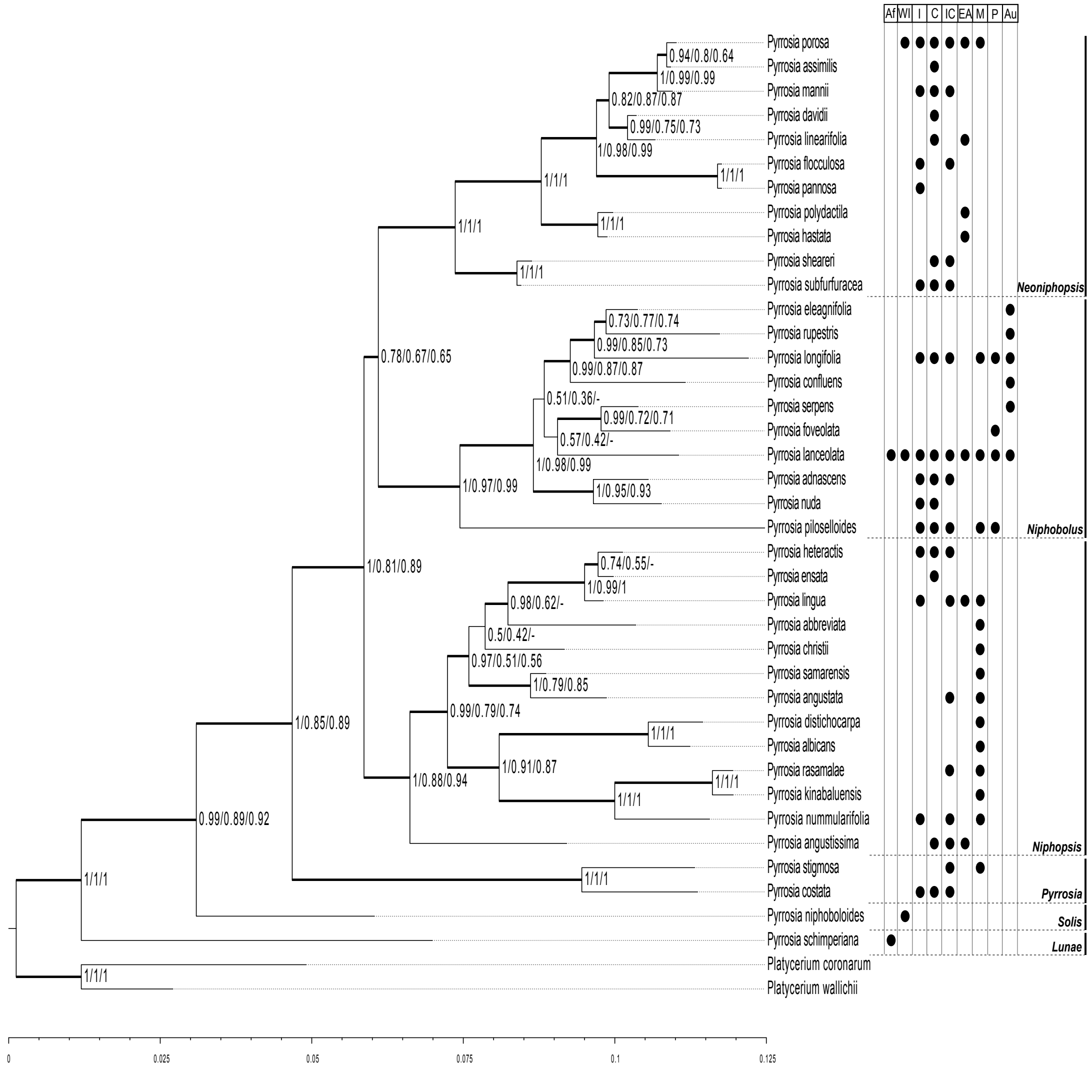

FIG. 1. Phylogenetic tree for *Pyrrosia* inferred by comparing sequences of DNA for the *rbcL* and *atpB* plastid genes, and for the *rps4-trnS* intergenic spacer. Values on branches indicate, respectively, Bayesian inference posterior probability, ML probability and MP probability (tree length = 848 steps, CI = 0.6368, HI = 0.3632 and RI = 0.7636). Bold branches indicate branches supported by both analyses (>50%). On right side, table indicates distribution range of each taxon (Af: Southern Africa; WI: Western Indian Ocean; I: Indian Subcontinental; C: China; IC: Indo-China; EA: Eastern Asia; M: Malesia; P: Papuasia; and Au: Australasia), followed by subgenera proposed here.

2012), the best-fit nucleotide substitution model for the whole set of three markers including missing data was calculated, resulting in the TPM2+I+G model.

A Bayesian inference was conducted using MrBayes v.3.2.5 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003), under the MCMC method for 1000000 (1 million) generations. Priors were set as equal, and the posterior was sampled at each 1000 generations, being the first 25% discarded as *burn-in*. For the Maximum Likelihood analysis, trees were generated using RAxML (Stamatakis 2006), over 1000 bootstrap replications. Also, using PAUP* v.4 (Swofford 2003), phylogenetic trees were heuristically drawn under a Maximum Parsimony (MP) criterion and

after that 1000 bootstrap replicates were performed. The acquired trees were edited using FigTree v.1.4.2 (Rambaut & Drummond 2012).

Subgenera delimitation

Distribution and morphological data for the species analyzed were acquired from previous studies on the genus (Ching 1933, 1935, Hovenkamp 1984, 1986, Shing 1983, Shing & Iwatsuki 1997) and from personal observations. The traits were optimized to the acquired phylogeny by using Mesquite v.3.04 (Maddison & Maddison 2015). The results were used to delimit subgenera and to construct the identification key and descriptions. The geographic distribution for the proposed subgenera is based on the system of Brummitt *et al*. (2001).

Results

Sequence analysis

By combining data available at GenBank with the newly acquired sequences, 38 different species of *Pyrrosia* plus two species of *Platycerium* were analyzed (Appendix 1). Based on the classification proposed by Hovenkamp (1986), the sampling of species covered around 65% of the species in the genus. In total, 2154 bp were analyzed in the final dataset. Sequences for some of the unsampled species were available in GenBank and were included in the analysis (Appendix 1). For some of the species, sequences for the three markers investigated were unavailable. In those cases the missing sequences were treated as missing data (Appendix 3). A tree based on the dataset excluding taxa with missing data is shown in Appendix 4 for reference. The discussion below focuses on the tree containing the highest number of taxa (*i.e.* including missing data).

Phylogenetic analysis

The resulting phylogenetic tree for the Bayesian inference is shown in Fig. 1, with the Bayesian inference posterior probabilities, ML and MP supports associated with each node and the distribution ranges categorized according to Brummitt *et al*. (2001). The MP analysis resulted in a single tree with a length of 848 steps, CI = 0.6368, HI = 0.3632 and RI = 0.7636. The overall topology of the tree indicates that the species are grouped in six different lineages, of which the two most basal ones are represented by African and Madagascan species.

Discussion

Subgeneric grouping

The acquired tree's topology indicates that many of the groups circumscribed by previous classifications (Hovenkamp 1986, Shing & Iwatsuki 1997) are not monophyletic, with representatives scattered throughout the phylogeny (Table 1, Fig. 1). Based on the acquired phylogeny, only one of the 10 groups proposed by Hovenkamp (1986), the *albicans* group, can still be considered as possibly monophyletic. The *albicans* group *sensu* Hovenkamp (1986) consists of six species: *Pyrrosia albicans* Ching, *P. asterosora* (Baker) Hovenkamp, *P. distichocarpa* (Mett.) K.H. Shing, *P. kinabaluensis* Hovenkamp, *P. nummulariifolia* (Sw.) Ching and *P. rasamalae* (Racib.) K.H. Shing (Table 1). Five of these six species are included in the phylogenetic tree and emerged as a monophyletic clade (Fig. 1).

The *costata* group and *africana* group was insufficiently sampled in this analysis and further conclusions about its monophyly are impossible. The *costata* group *sensu* Hovenkamp (1986) includes five species, *Pyrrosia costata* (Wall. ex C.Presl) Tagawa & K. Iwats., *P. platyphylla* Hovenkamp, *P. princeps* (Mett.) C.V. Morton, *P. splendens* Ching and *P. stigmosa*, of which *P. costata* and *P. stigmosa* were examined in this phylogenetic analysis. The *africana* group *sensu* Hovenkamp (1986), represented by *P. africana* Ballard and *P. schimperiana* (Kuhn) Alston, corresponds to the most basal lineage of the genus. In this study, one African and one Madagascan species were sampled and both appeared as basal lineages in the acquired phylogenetic tree (Fig.1), suggesting that they are the most basal extant group in the genus. Still, other African species

TABLE 1. Comparison of assignment of species to infrageneric groups of *Pyrrosia* in this study, by Hovenkamp (1986) and Shing & Iwatsuki (1997). Asterisks indicate species not included in present phylogenetic analysis.

Species	Present study	Hovenkamp (1986)	Shing & Iwatsuki (1997)
Pyrrosia costata	Subg. *Pyrrosia*	*costata* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Heteractides*
*P. platyphylla**		*costata* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Costae*
*P. princeps**		*costata* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Heteractides*
*P. splendens**		*costata* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. stigmosa		*costata* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Costae*
*P. africana**	Subg. *Lunae*	*africana* group	N/A
P. schimperiana		*africana* group	N/A
P. assimilis	Subg. *Neoniphopsis*	*porosa* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Drakeanae*
*P. boothii**		*sheareri* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. davidii		N/A	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Drakeanae*
*P. drakeana**		*sheareri* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. flocculosa		*sheareri* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Costae*
*P. gardneri**		N/A	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. hastata		*sheareri* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. linearifolia		*porosa* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. mannii		N/A	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. pannosa		N/A	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
*P. penangiana**		N/A	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Drakeanae*
P. polydactyla		*sheareri* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Drakeanae*
P. porosa		*porosa* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. sheareri		*sheareri* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. subfurfuracea		*sheareri* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. adnascens	Subg. *Niphobolus*	N/A	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
*P. ceylanica**		*lanceolata* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. confluens		*confluens* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. eleagnifolia		*confluens* group	N/A
*P. fallax**		*lanceolata* group	N/A
P. foveolata		*lanceolata* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. lanceolata		*lanceolata* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. longifolia		*lanceolata* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
*P. novoguineae**		*angustata* group	N/A
P. nuda		*lanceolata* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. piloselloides		*piloselloides* group	N/A
P. rupestris		*confluens* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. serpens		*confluens* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. abbreviata	Subg. *Niphopsis*	*lingua* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. albicans		*albicans* group	N/A
P. angustata		*angustata* group	Subgenus *Niphobolus*
P. angustissima		*lingua* group	N/A
*P. asterosora**		*albicans* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. christii		*lingua* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Costae*
P. distichocarpa		*albicans* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. ensata		N/A	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. heteractis		N/A	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Heteractides*
*P. heterophylla**		*piloselloides* group	N/A
P. kinabaluensis		*albicans* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Costae*
*P. laevis**		N/A	N/A
P. lingua		*lingua* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. nummulariifolia		*albicans* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
*P. petiolosa**		*lingua* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
P. rasamalae		*albicans* group	Subgenus *Pyrrosia*, Sect. *Dichlamys*, Ser. *Porosae*
P. samarensis		*angustata* group	Subgenus *Niphobolus*
*P. sphaerosticha**		*lingua* group	Subgenus *Pyrrosia*, Sect. *Pyrrosia*, Ser. *Pyrrosia*
*P. avaratra**	Subg. *Solis*	N/A	N/A
P. niphoboloides		*piloselloides* group	N/A
*P. rhodesiana**		*porosa* group	N/A
*P. stolzii**		*porosa* group	N/A

were not evaluated herein, and further conclusions should be made with care. By observing the topology of the phylogeny (Fig. 1) and the morphological traits of the species in each lineage, we propose that the genus should be divided into six subgenera (Table 1).

Distribution

The first two groups that emerge in the tree are *Pyrrosia schimperiana* and *P. niphoboloides* (Luerss.) M.G. Price, which exclusively occur in Africa and Madagascar, respectively. *Pyrrosia schimperiana* was included in the *P. africana* group in Hovenkamp's classification (1986), while *P. niphoboloides* was included in the *piloselloides* group, together with *P. heterophylla* (L.) M.G. Price and *P. piloselloides* (L.) M.G. Price. This topology, showing two basal groups with African distribution gives support to the hypothesis of an African origin for the *Pyrrosia* and its sister group *Platycerium* (Janssen *et al*. 2007). Kreier & Schneider (2006) hypothesized an African origin for *Platycerium*, but found difficulty in supporting this hypothesis due to the variability of features and lack of critical characteristics in *Pyrrosia*, as well as the lack of access to samples of African groups of *Pyrrosia* (Kreier & Schneider 2006). We propose that the two taxa at the base of the acquired tree represent two different subgeneric lineages within *Pyrrosia*.

The remaining clades include the majority of species that we classify into four different subgenera. When comparing the ranges of these subgenera, a distributional predominance can be observed. Subgenus *Pyrrosia* occurs in India, China and the Malesian region; subgenus *Niphopsis* has a predominant distribution in the Eastern Asian/Malesian regions; subgenus *Niphobolus* is represented both on the India subcontinent/Chinese regions and in the Papuasian/Australian regions; and subgenus *Neoniphopsis* has a distribution ranging from the western Indian Ocean to eastern Asia (Fig. 1). Nevertheless, some of the species within each group have a broader distribution, such as *Pyrrosia lanceolata* (L.) Farw., the most widely distributed species.

Hovenkamp (1986) based his biogeographical discussion on three groups, the *costata* group (herein subgenus *Pyrrosia*), the *lingua* group and the *angustata* group (both in subgenera *Niphobolus* and *Niphopsis*). According to Hovenkamp (1986), the ancestors of both the *costata* group and the *lingua* group should have been present on the Indian subcontinent, corresponding to the breakup of Gondwana, before it came in close contact with the Southeast Asian region. According to Hovenkamp (1986, pp. 119–120), some groups would have reached Africa, scattering through the continent and the Madagascan region.

The present study suggests that the ancestor of the genus originated in Africa, later dispersing through other regions of the globe. This dispersion could have happened through drifting of the Indian subcontinent, followed by long distance dispersal events towards the Malesian region, Papuasia and the Pacific region. Alternatively, the Pacific distribution could have resulted from a later collision of Papua-Australia and Southeast Asia (Hovenkamp 1986, p. 120).

The wide distribution of some species (*e.g. Pyrrosia lanceolata*), however, is still not explained. Within *P. lanceolata*, Hovenkamp (1986) recognized six "entities," indicating *P. lanceolata* may be a complex of morphologically similar species requiring further taxonomic investigation.

Morphological traits

Traits important for the identification of the subgenera are included in the identification key and in Table 2. The morphological traits of species in the most basal African clades allow us to hypothesize that the lineages within *Pyrrosia* diverged from predominantly short-creeping, rhizomatous plants with sessile fronds without sclerenchyma and with polocytic stomata. Derivative traits include the long creeping rhizome, increased hydathodes of the lamina and the emergence of boat shaped rays on the trichomes.

The clade composed by *Pyrrosia stigmosa* and *P. costata* (herein subgenus *Pyrrosia*), has rhizomes with basifixed scales and a reduced number of annulus cells in the sporangia, condi-

tions that do not occur in any other clade. The reduction in the number of annulus cells was explored by Hovenkamp (1986), who used it as a key trait for recognizing the *costata* group. Although our analysis includes only two of the five species of the *costata* group, the separation of the *P. stigmosa-costata* clade suggests the reliability of Hovenkamp's grouping and the evolutionary relevance of the reduced annulus in the evolution of *Pyrrosia*.

The remaining clades are plesiomorphic for scale insertion and the number of annulus cells and are subdivided here into three subgenera *Neoniphopsis*, *Niphobolus* and *Niphopsis*. Subgenera *Niphobolus* and *Niphopsis*, which did not form a monophyletic group in our analysis, share several common morphological traits, such as long creeping rhizomes with a ventral groove, dimorphic leaves in many species and fronds with a distinct stipe. These traits are not shared with the remaining subgenus *Neoniphosis* (Table 2).

Taxonomic treatment

Pyrrosia Mirb., Hist. Nat. Gen. 4 (1803)

Type: *Pyrrosia chinensis* Mirb. (=*Pyrrosia stigmosa* (Sw.) Ching).

Rhizome short to long creeping, perforated-dictyostelic. **Roots** dense in short-creeping species and in turfs in long-creeping species. **Scales** appressed, basifixed or peltate, varying from pale to dark brown, margins entire, ciliate or denticulate. **Fronds** usually simple, lobed or dissected in few species, non-stipitate or stipitate, linear to oblanceolate, succulent in some species, mono- or dimorphic. **Indumentum** corresponding to stellate hairs, varying from whitish to blackish; axis of hairs boat shaped or acicular. **Hydathodes** present or not, usually sunken on adaxial surface of the lamina when present, scattered over the lamina or organized in rows near margins. Veins anastomosing, forming areoles by connection of secondary **veins** through tertiary veins; veinlets free, included or excluded in areoles. **Sori** round, usually on distal portion of the lamina, superficial or sunken, varying in size and number, sometimes confluent into coenosori. **Paraphyses** present or absent. **Annuli** of few to several cells.

1. Subgenus **Lunae** Vasques **subg. nov**. —Fig. 2, A–B

Similar to subgenus Solis, but differing by the polocytic stomata surrounded by moon-shaped subsidiary cells, monomorphic indumentum with wool-like hairs and entire scales on the rhizome. Distributed exclusively in continental Africa.

Key to the subgenera of *Pyrrosia*

1a. Madagascar or continental Africa 2
1b. Indian subcontinent, China, Indo-China, Eastern Asia, Malesian region, Papuasia or Australasia 3
2a. Stomata polocytic; indumentum monomorphic; scales entire (rarely dentate-ciliate) .... **1. Subgenus *Lunae***
2b. Stomata pericytic; indumentum dimorphic or monomorphic; scales dentate-ciliate or entire...... **6. Subgenus *Solis***
3a. Scales of rhizome basifixed; sporangial annuli reduced, consisting of less than 12 cells, not extending to stalk .... **5. Subgenus *Pyrrosia***
3b. Scales of rhizome peltate or pseudopeltate; sporangial annuli consisting of more than 12 cells, extending to stalk... 4
4a. Rhizome short-creeping .... **2. Subgenus *Neoniphopsis***
4b. Rhizome long-long creeping 5
5a. Fronds monomorphic 6
5b. Fronds dimorphic 7
6a. Fronds linear; phyllopodia covered by scales (Fig. 4G) .... **3. Subgenus *Niphobolus* (*P. longifolia*)**
6b. Frond lanceolate, linear or oblong; phyllopodia not covered by scales .... **4. Subgenus *Niphopsis***
7a. Sclerenchyma strands in rhizomes central 8
7b. Sclerenchyma strands in rhizome peripheral or dispersed .... **4. Subgenus *Niphopsis***
8a. Indumentum monomorphic .... **3. Subgenus *Niphobolus***
8b. Indumentum dimorphic .... **4. Subgenus Niphopsis (*P. nummulariifolia*)**

TABLE 2. Comparison of traits among subgenera.

Subgenus	*Lunae*	*Neoniphopsis*	*Niphobolus*	*Niphopsis*	*Pyrrosia*	*Solis*
Rhizome habit	short	short (long in *P. linearifolia*)	long	long	short	short (long in *P. niphoboloides*)
Rhizome features	terete	terete (ventrally grooved in *P. linearifolia*)	ventrally grooved (terete in *P. piloselloides* and *P. longifolia*)	ventrally grooved or	terete	terete
Schlerenchyma strands	absent	present (absent in *P. pannosa*)	present (absent in *P.eleagnifolia* and *P. serpens*)	present (absent in *P. christii* and *P. kinabaluensis*)	absent	absent (present in *P. stolzii*)
Scales on rhizome - insertion	pseudopeltate	peltate or pseudopeltate	peltate	peltate	basifixed	peltate
Scales on rhizome - margins	entire to ciliate-dentate	irregularly cilliate-dentate	cilliate-dentate (entire in *P. ceylanica* and *P. longifolia*)	entire or ciliate-dentate	cilliate-dentate (entire in *P. splendens*)	cilliate-dentate
Stipe	absent to indistinct	absent or present	present (absent in *P. longifolia*)	present	present	absent (present in *P. niphoboloides*)
Frond	monomorphic	monomorphic	dimorphic (monomorphic in *P. longifolia*)	monomorphic or dimorphic	monomorphic	monomorphic (dimorphic in *P. niphoboloides*)
Indumenta	monomorphic	mono or dimorphic	monomorphic	mono or dimorphic	dimorphic (monomorphic in *P. stigmosa*)	monomorphic (dimorphic in *P.niphoboloides*)
Hair rays	acicular or boat-shaped	acicular or boat-shaped	boat-shaped	acicular or boat-shaped	acicular or boat-shaped	acicular
Hydathodes	absent(*P. schimperiana*) or present (*P. africana*)	scattered over surface of lamina	absent or in a singular row	absent or scattered over surface of lamina	constant and scattered	present (absent in *P. niphoboloides* and *P. rhodesiana*)
Stomata - insertion	superficial or sunken	superficial or sunken	sunken	sunken (superficial in *P. distichocarpa*)	superficial or sunken	sunken
Stomata - kind	polocytic	pericytic or polocytic	pericytic	pericytic	pericytic	pericytic
Sporangium annulus	extending to the stalk	extending to the stalk	extending to the stalk	extending to the stalk	reduced	extending to the stalk
Paraphyses	absent	absent	mostly present	mostly absent	absent	absent

Type: *Pyrrosia schimperiana* (Kuhn) Alston

Rhizome short creeping, terete, sclerenchyma strands absent. **Scales** pseudopeltate, margins entire to ciliate-dentate. **Fronds** monomorphic, sessile or indistinctly stipitate lanceolate, base gradually narrowed, apex acute to acuminate. **Indumentum** monomorphic, rays of hairs acicular to boat shaped. **Hydathodes** absent, in a singular row or scattered over the lamina. **Stomata** sunken to superficial, polocytic. **Sori** round, numerous, with indument similar to indumentum on sterile lamina. **Annulus** of 12 cells or more, extending to stalk. **Paraphyses** absent.

Included species. *Pyrrosia africana* Ballard* and *P. schimperiana* (Kuhn) Alston. Asterisk (*) indicates species not examined in the present molecular phylogenetic analysis, but which share morphological characteristics with other included species. Asterisks are applied in the same way in the following subgenera.

2. Subgenus **Neoniphopsis** (Nakai) Vasques **comb. & stat. nov.** —Fig. 3

Neoniphopsis Nakai, Bot. Mag. Tokyo 42: 217 (1928) – Type: *Neoniphopsis linearifolia* (Hook.) Nakai (= *Pyrrosia linearifolia* (Hook.) Ching).
Pyrrosia sect. *Dichlamys* Ching & Shing, Amer. Fern J. 73: 77 (1983) – Type: *Niphobolus mollis* Kunze (= *Pyrrosia porosa* (C. Presl) Hovenkamp).
Pyrrosia ser. *Molles* Ching & Shing, Amer. Fern J. 73: 77 (1983) – Type: *Niphobolus mollis* Kunze (= *Pyrrosia porosa* (C. Presl) Hovenkamp).

Rhizome short creeping and terete, or long creeping and ventrally grooved, sclerenchyma strands scattered throughout or absent. **Scales** peltate or pseudopeltate, irregularly ciliate-dentate. **Fronds** stipitate or sessile, monomorphic; linear, lanceolate, elliptic-lanceolate, ovate, hastate or pedately dissected, base gradually narrowed, cuneate, truncate or cordate, sometimes decurrent, apex acute, acuminate, obtuse or rounded. **Indumentum** mono- or dimorphic, rays

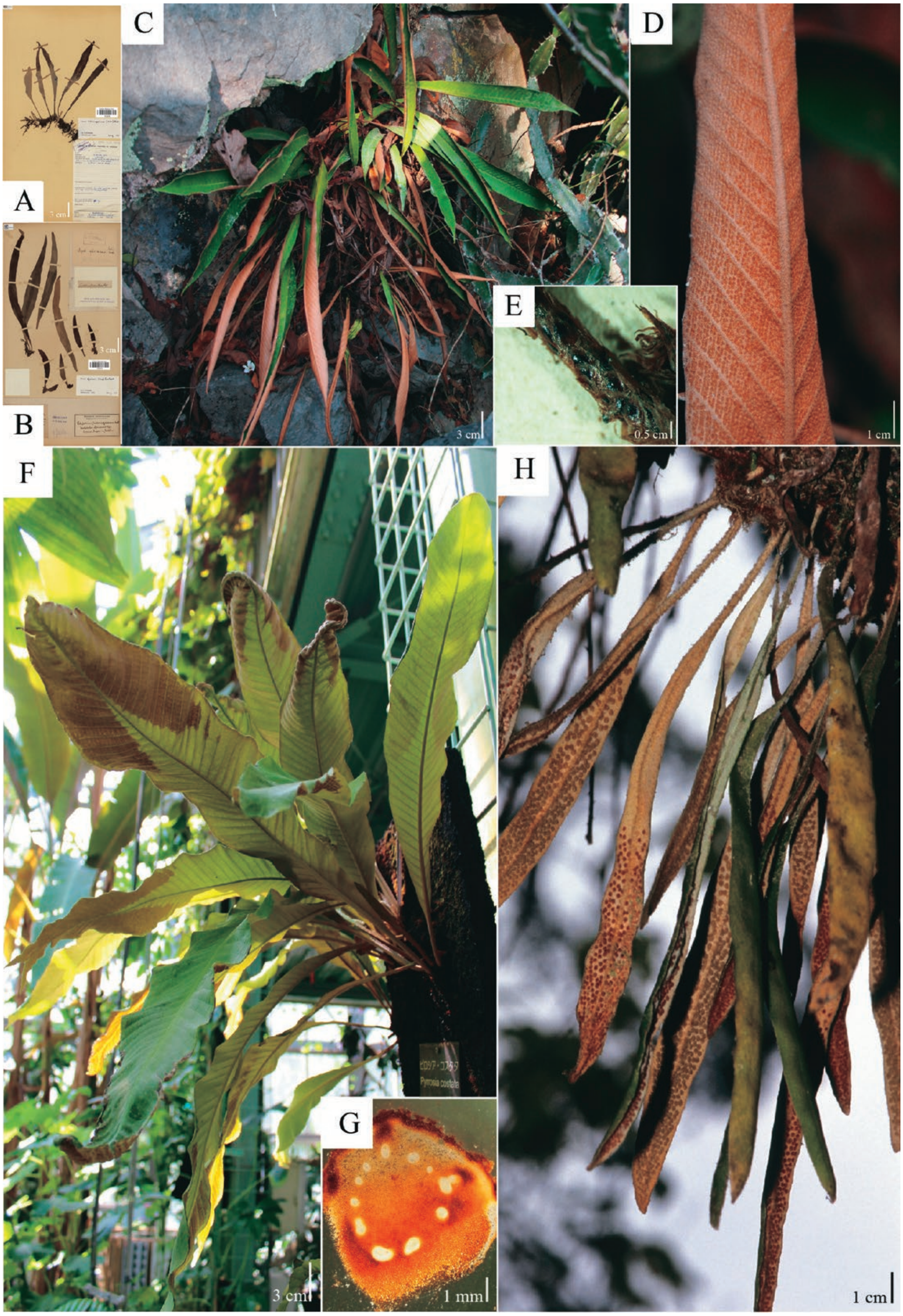

FIG. 2. Representatives of subgenus *Lunae* (A–B), *Pyrrosia* (C–G) and *Solis* (H). Photographers are credited within brackets. A. *Pyrrosia schimperiana*: voucher [G. Rouhan]; B. *Pyrrosia africana*: voucher [G. Rouhan]; C–E. *Pyrrosia stigmosa*. C, short creeping habit; D, abaxial surface of lamina showing round and numerous sori; E, scales appressed on rhizome [S. Tagane & D. T. Vasques]. F–G. *Pyrrosia costata*. F, short-creeping habit; G, non-sclerenchymatous rhizome anatomy [D. T. Vasques]; H. *Pyrrosia rhodesiana*: short-creeping habit [G. Rouhan].

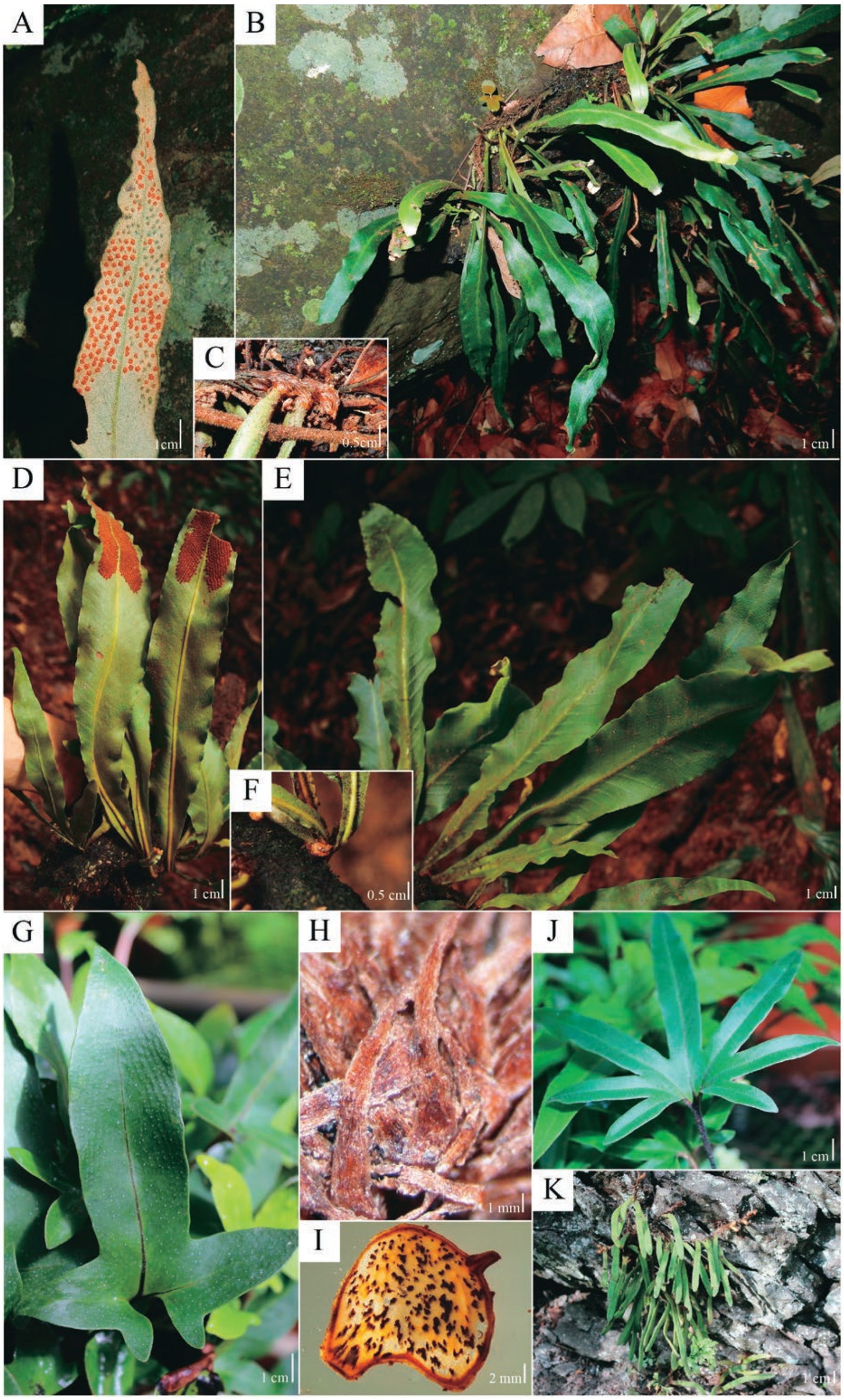

FIG. 3. Representatives of subgenus *Neoniphopsis*. Photographers are credited within brackets. A–C. *Pyrrosia porosa*. A, abaxial surface of lamina showing round sori; B, short-creeping habit; C, petiole insertion point bearing scales [H. Kanemitsu]. D–F. *Pyrrosia subfurfuracea*. D, abaxial surface of lamina with distal sori; E, short-creeping habit; F, petiole insertion point bearing scales [S. Tagane]. G–I. *Pyrrosia hastata*. G, adaxial surface of hastate lamina with hydathodes; H, rhizome scales; I, rhizome anatomy showing several strands of schlerenchyma [D. T. Vasques]. J. *Pyrrosia polydactyla*: adaxial lamina [D. T. Vasques]. K. *Pyrrosia linearifolia*: short-creeping habit [D. T. Vasques].

acicular or boat shaped. **Hydathodes** scattered over lamina. **Stomata** superficial or sunken, pericytic or polocytic. **Sori** round, superficial, indumentum similar to indumentum on lamina. **Annulus** of 12 cells or more, extending to the stalk. **Paraphyses** absent.

Subgenus *Neoniphopsis* includes species from western India to eastern Asia. The species referable to this subgenus include *Pyrrosia hastata* and *P. polydactyla* (Hance) Ching with hastate/pedately dissected leaves, *P. sheareri* (Baker) Ching with basally lobed leaves, *P. mannii* (Giesenh.) Ching and *P. pannosa* (Mett. ex Kuhn) Ching.

Included species. *Pyrrosia assimilis* (Baker) Ching, *P. boothii** (Hook.) Ching, *P. davidii* (Giesenh. ex Diels) Ching, *P. drakeana** (Franch.) Ching, *P. flocculosa* (D. Don) Ching, *P. gardneri** (Mett.) Sledge, *P. hastata* (Houtt.) Ching, *P. linearifolia* (Hook.) Ching, *P. mannii* (Giesenh.) Ching, *P. pannosa* (Mett. ex Kuhn) Ching, *P. penangiana** Holttum, *P. polydactyla* (Hance) Ching, *P. porosa* (C. Presl) Hovenkamp, *P. sheareri* (Baker) Ching and *P. subfurfuracea* (Hook.) Ching.

3. Subgenus **Niphobolus** (Kaulf.) Vasques **comb. nov.** —Fig. 4

Niphobolus Kaulf., Enum. Filic. 124 (1824) – *Polypodium* subgenus *Niphobolus* (Kaulf.) Hook, Sp. Fil. (1863) 43 – Lectotype: *Niphobolus adnascens* (Sw.) Kaulf. (= *Pyrrosia lanceolata* (L.) Farw.) (designated by J. Smith, 1857).
Candollea Mirb., Hist. Nat. Gen. 3: 471 (1803) – Lectotype: *Candollea longifolia* (Burm. f.) Mirb. (= *Pyrrosia longifolia* (Burm. f.) C.V. Morton) (designated by C.V. Morton, 1946).
Cyclophorus Desv., Berl. Mag. 5: 300 (1811) – Type: *Cyclophorus adnascens* (Sw.) Desv.
Drymoglossum C. Presl, Tent. Pter. 227 (1836) – Type: *Drymoglossum piloselloides* (L.) Presl (= *Pyrrosia piloselloides* (L.) M.G. Price).

Rhizome long creeping, terete or ventrally grooved, strands of sclerenchyma none or few and centrally distributed. **Scales** peltate, with structures called annular fingers on cells (Hovenkamp 1986), margin irregularly ciliate-dentate or entire. **Fronds** stipitate or sessile, mono- or dimorphic, linear or lanceolate, base gradually narrowed, cuneate or attenuate, apex acute, acuminate, apiculate, truncate, obtuse or rounded. **Indumentum** monomorphic, with boat shaped rays. **Hydathodes** absent or present in a singular row. **Stomata** sunken, pericytic. **Sori** round. **Annulus** of 12 cells or more, extending to stalk. **Paraphyses** mostly present.

We include *Pyrrosia foveolata* (Alston) C.V. Morton, whose position remained unresolved by Hovenkamp (1986), in subgenus *Niphobolus*. *Pyrrosia adnascens* (Sw.) Ching was regarded as a subspecies within *P. lanceolata* by Hovenkamp (1986), but appears in a separate clade in our phylogeny, indicating that the two should be treated as distinct species.

Included species. *Pyrrosia adnascens* (Sw.) Ching, *P. ceylanica** Sledge, *P. confluens* (R. Br.) Ching, *P. eleagnifolia* (Bory) Hovenkamp, *P. fallax** M.G. Price, *P. foveolata* (Alston) C.V. Morton, *P. lanceolata* (L.) Farw., *P. longifolia* (Burm. f.) C.V. Morton, *P. novoguineae** (Christ) M.G. Price, *P. nuda* (Giesenh.) Ching, *P. piloselloides* (L.) M.G. Price, *P. rupestris* Ching and *P. serpens* (G. Forst.) Ching.

4. Subgenus **Niphopsis** (J. Sm.) Shing —Fig. 5

Pyrrosia subgenus *Niphopsis* (J. Sm.) Shing, Amer. Fern J 73: 77 (1983) – *Niphopsis* J. Sm., Cat. Cult. Ferns 6 (1857) – Type: *Niphopsis angustata* (Sw.) J. Sm. (= *Pyrrosia angustata* (Sw.) Ching).
Pteropsis Desv., Mém. Soc. Linn. Par. 6: 218 (1827) – Lectotype: *Acrostichum heterophyllum* L. (= *Pyrrosia heterophylla* (L.) M.G. Price) (designated by Pichi Sermolli, 1953).
Polycampium C. Presl, Abh. Königl. Böhm. Ges. Wiss., ser. 5 6: 495–496 (1851) – Lectotype: *Polycampium lingua* (Thunb.) C. Presl (= *Pyrrosia lingua* (Thunb.) C. Presl) (designated by C. Christensen, 1906).
Galeoglossa C. Presl, Abh. Königl. Böhm. Ges. Wiss., ser. 5 6: 495–496 (1851) – Lectotype: *Galeoglossa nummulariifolia* (Sw.) C. Presl (= *Pyrrosia nummulariifolia* (Sw.) Ching) (designated by C. Christensen, 1906).
Saxiglossum Ching, Contr. Inst. Bot. Nat. Acad. Peiping 2: 5-6 (1933) – Type: *Saxiglossum taenioides* (C. Chr.) Ching (= *Pyrrosia angustissima* (Giesenh. ex Diels) Tagawa & K. Iwats.).
Pyrrosia ser. *Heteractides* Ching & Shing, Amer. Fern J. 73: 77 (1983) – Type: *Polypodium heteractis* Mett. ex Kuhn (= *Pyrrosia heteractis* (Mett. ex Kuhn) Ching).

Rhizome long creeping, terete or ventrally grooved, schlerenchyma strands centrally or peripherally distributed or absent. **Scales** peltate, margins entire or dentate-ciliate. **Fronds** stipitate, mono- or dimorphic, linear, lanceolate or oblong, base cuneate, truncate or cordate, sometimes decurrent, apex acute, acuminate, obtuse or rounded. **Indumentum** mono- or dimorphic, with acicular or boat shaped rays. **Hydathodes** absent in most species or present and scattered over the lamina. **Stomata** sunken or superficial, pericytic. **Sori** round. **Annulus** of 12 cells or more, extending to the stalk. **Paraphyses** mostly absent.

Pyrrosia subgenus *Niphopsis* includes species from Indian, Chinese and Malesian regions. *Pyrrosia angustissima* (Giesenh. ex Diels) Tagawa & K. Iwats. (not included in any group by Hovenkamp (1986), who indicated that it was possibly related to the angustata group) is here assigned to subgenus *Niphopsis*. *Pyrrosia heteractis* (Mett. ex Kuhn) Ching, sometimes treated as a variety of *P. lingua* (Thunb.) Farw. (Hovenkamp 1986), but recognized as a distinct species by others (Shing & Iwatsuki 1997), appears separated from *P. lingua,* indicating that they represent distinct species.

Included species. *Pyrrosia abbreviata* (Zoll. & Moritzi) Hovenkamp, *P. albicans* Ching, *P. angustata* (Sw.) Ching, *P. angustissima* (Giesenh. ex Diels) Tagawa & K. Iwats., *P. asterososa** (Baker) Hovenkamp, *P. christii* (Giesenh.) Ching, *P. distichocarpa* (Mett.) K.H. Shing, *P. ensata* Ching ex K.H. Shing, *P. heteractis* (Mett. ex Kuhn) Ching, *P. heterophylla** (L.) M.G. Price, *P. kinabaluensis* Hovenkamp, *P. laevis** (J. Sm. ex Bedd.) Ching, *P. lingua* (Thunb.) Farw., *P. nummulariifolia* (Sw.) Ching, *P. petiolosa** (Christ) Ching, *P. rasamalae* (Racib.) K.H. Shing, *P. samarensis* (C. Presl) Ching and *P. sphaerosticha** (Mett.) Ching.

5. Subgenus **Pyrrosia** —Fig. 2, F–G

Apalophlebia C. Presl, Abh. Königl. Böhm. Ges. Wiss., ser. 56: 495–496 (1851) – Lectotype: *Apalophlebia venosa* (Blume) C. Presl (= *Pyrrosia stigmosa* (Sw.) Ching (designated by C. Christensen, 1906).

Pyrrosia ser. *Costatae* Ching & Shing, Amer. Fern J. 73: 77 (1983) – Type: *Apalophlebia costata* C. Presl (= *Pyrrosia costata* (C. Presl) Tagawa & K.Iwats.).

Rhizome short-creeping, terete, sclerenchyma strands absent. **Scales** basifixed, with thin walled cells, margins irregularly ciliate-dentate or entire, bearing glands at base. **Fronds** stipitate, monomorphic, lanceolate, base gradually narrowed or cuneate, apex acute to acuminate or sometimes rounded or obtuse. **Indumentum** mono- or dimorphic, composed of hairs with acicular or boat shaped rays. **Hydathodes** consistently present, scattered over adaxial surface of lamina. **Stomata** superficial or sunken, pericytic. **Sori** round, superficial on lamina. **Annulus** of fewer than 12 cells, not extending to the stalk. **Paraphyses** absent.

Some species of subgenus *Pyrrosia* share a reduction in the number of annulus cells to a small apical part (Hovenkamp 1986).

Included species. *Pyrrosia costata* (Wall. ex C. Presl) Tagawa & K. Iwats., *P. platyphylla** Hovenkamp, *P. princeps** (Mett.) C.V. Morton, *P. splendens** Ching and *P. stigmosa* (Sw.) Ching.

6. Subgenus **Solis** Vasques **subg. nov.** —Fig. 2, H

Plants bearing sunken and pericytic stomata, with guard cells surrounded by a round subsidiary cell.

Type: *Pyrrosia niphoboloides* (Luerss.) M.G. Price

Rhizome short to long creeping, terete; sclerenchyma strands absent to more than 5. **Scales** peltate, margins entire to ciliate-dentate. **Fronds** stipitate or sessile, monomorphic or rarely dimorphic, ovate to lanceolate, base rounded to short attenuate, apex rounded. **Indumentum** dimorphic, rarely monomorphic, rays of hairs acicular. **Hydathodes** in a marginal row or scattered over the lamina or absent. **Stomata** sunken, pericytic. **Sori** round, numerous, coenosoral in some species, with indumentum similar to indumentum on sterile lamina. **Annulus** or 12 cells or more, extending to stalk. **Paraphyses** absent.

Included species. *Pyrrosia avaratra** Rakotondr. & Hovenkamp, *P. niphoboloides* (Luerss.) M.G. Price, *P. rhodesiana** (C. Chr.) Schelpe and

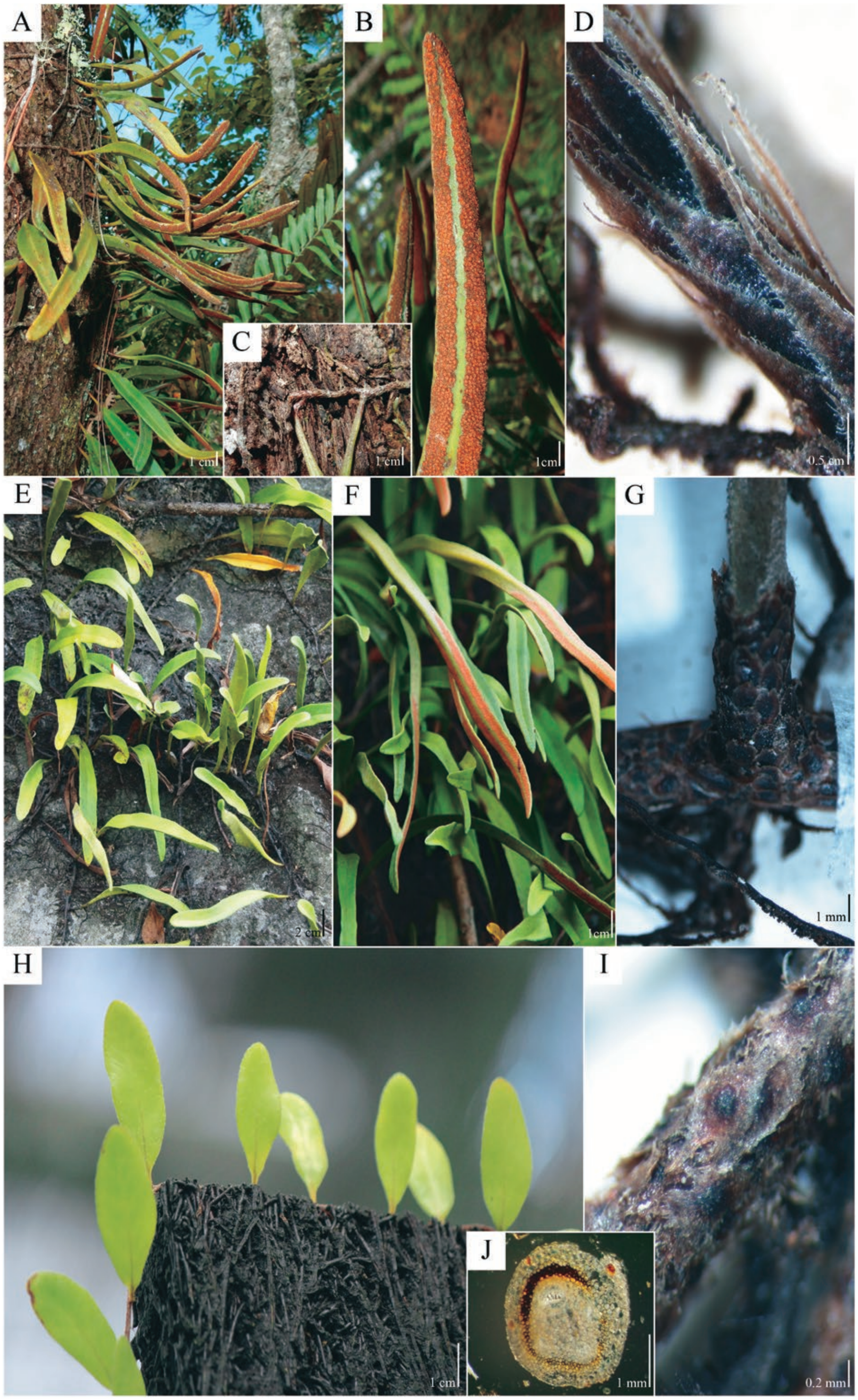

FIG.4. Representatives of subgenus *Niphobolus*. Photographers are credited within brackets. A–D. *Pyrrosia lanceolata*. A, long-creeping habit; B, abaxial surface of lamina showing numerous sori; C, rhizome detail; D, rhizome's scales with dentate cilia [S. Tagane & D. T. Vasques]. E–G. *Pyrrosia longifolia*. E, long-creeping habit; F, fertile lamina with distal sori; G, petiole insertion point bearing scales [S. Tagane & D. T. Vasques]. H–J. *Pyrrosia piloselloides*. H, long-creeping habit; I, rhizome scales with cilia; J, rhizome anatomy showing defined schlerenchyma ring [D. T. Vasques].

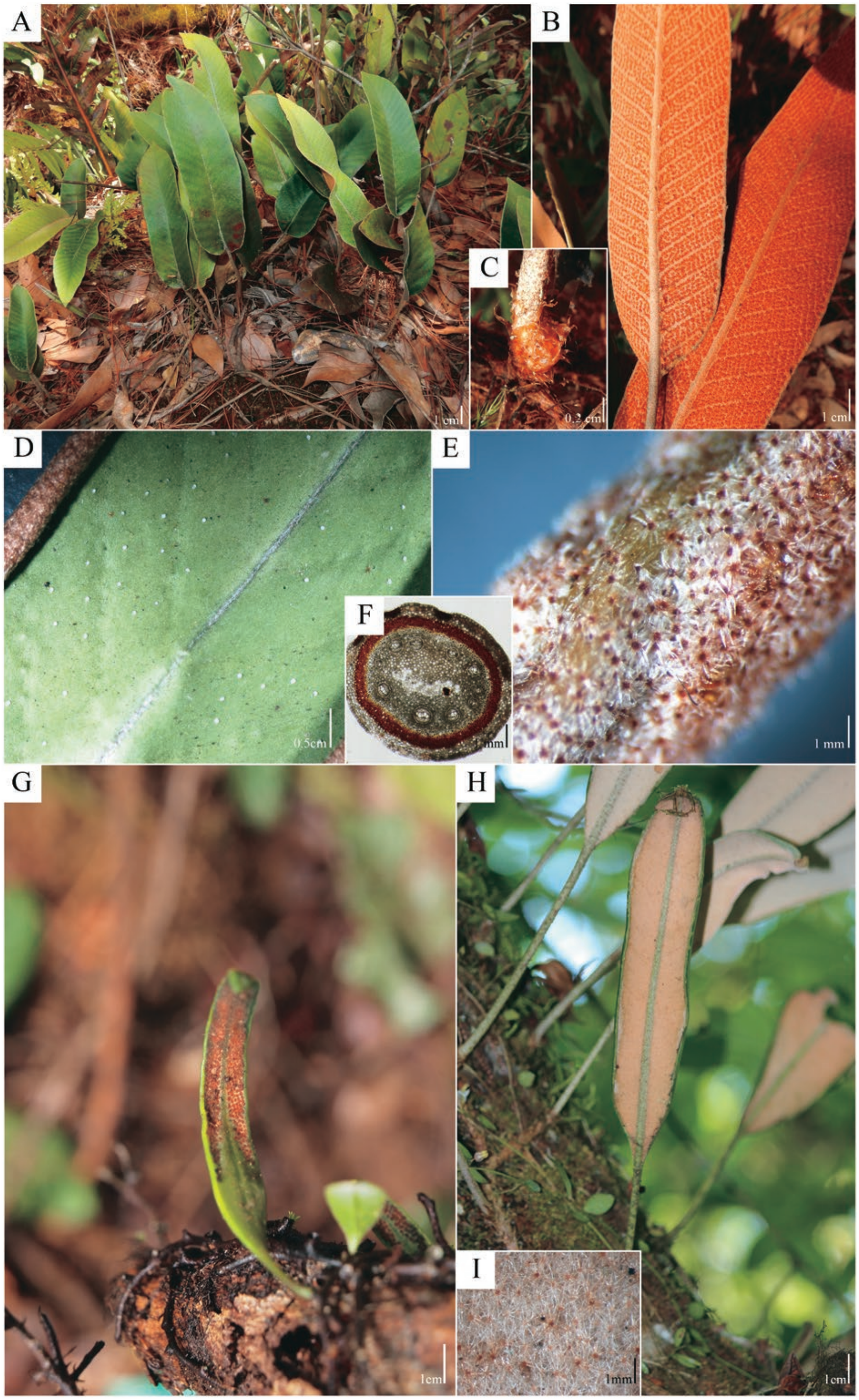

FIG.5. Representatives of subgenus *Niphopsis*. Photographers are credited within brackets. A–C. *Pyrrosia heteractis*. A, long-creeping habit; B, abaxial surface of lamina covered by sori; C, scales on rhizome at base of petiole [H. Kanemitsu]. D–F. *Pyrrosia lingua*. D, adaxial surface of lamina with hydathodes. E, petiole trichomes with boat-shaped rays; F, rhizome anatomy showing defined ring of schlerenchyma [D. T. Vasques]. G. *Pyrrosia rasamalae*. G, abaxial surface of lamina with sori [K. Fuse]. H–I. *Pyrrosia albicans*. H, abaxial surface of lamina densely covered by trichomes; I, trichomes on abaxial surface of lamina showing acicular rays [D. T. Vasques].

*P. stolzii** (Hieron.) Schelpe.

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Appendix 1. Information about sequences used in this study. Genera and species names are in bold-italic and italic, respectively. Names are followed by data for the three DNA markers analyzed (*rbcL*, *rps4-trnS* and *atpB*): voucher information, including place of collection and collector's name (for newly acquired data) or published date (GenBank data) and GenBank accession number.

Platycerium: *Platycerium coronarum* (Konig) Desv., Kim *et al*. (2013), JX103711, JX103669. Kreier, H.P. & Schneider, H. 2006, DQ164479. *Pl. wallichii* Hook., Kim *et al*. (2013), JX103728, JX103686. Kreier, H.P. & Schneider, H. 2006, DQ164492. ***Pyrrosia***: *P. abbreviata* (Zoll. & Moritzi) Hovenkamp., Indonesia, Sumatra, M. Ohkubo & T. Sano (TNS 1184179), KY064505, KY064531, KY064558. *P. adnascens* (Sw.) Ching, Ebihara *et al*. (2010), AB575294. *P. albicans* Ching, Indonesia, Sumatra, M. Ohkubo (TNS 1189776), KY064521, KY064553, KY064575. *P. angustata* (Sw.) Ching, Janssen *et al*. (2007), DQ642165, DQ642204. Malaysia, Penang Island, M. Ohkubo (TNS 1256467), KY064555. *P. angustissima* (Giesenh. Ex Diels) Tagawa & K. Iwats., China, Fujian, S.-J. Lin *et al*. (TNS 762131), KY064524, KY064552, KY064574. *P. assimilis* (Baker) Ching, China, Fujian, S.-J. Lin *et al*. (TNS 762151), KY064515, KY064554. P. christii (Giesenh.) Ching. Kreier & Schneider (2006), DQ164465, DQ164496. *P. confluens* (R. Br.) Ching, New Caledonia, Muzinger 1575 (P 02074), KY064528, KY064551, KY064576. *P. costata* (Wall. ex C. Presl) Tagawa & K. Iwats., Nepal, M. Ohkubo (TNS 1184172), KY064527, KY064550, KY064573. *P. davidii* (Giesenh. ex Diels) Ching, Ebihara *et al*. (2010), AB575295. *P. distichocarpa* (Mett.) K.H. Shing, Sumatra, Borastagi, M. Ohkubo & T. Sano (TNS 1184183), KY064520, KY064549, KY064572. *P. eleagnifolia* (Bory) Hovenkamp, Janssen *et al*. (2007), DQ642166, DQ642205. *P. ensata* Ching ex K.H. Shing, China, Bangsu, M. Kato (TNS 1184171), KY064523, KY064548, KY064571. *P. flocculosa* (D.Don) Ching, Bhutan, M. Ohkubo (TNS 1184173), KY064513, KY064538, KY064564. *P. foveolata* (Alston) C.V. Morton, Janssen *et al*. (2007), DQ642167, DQ642206. *P. hastata* (Thunb.) Ching, Japan, Kouichi (TNS 763873), KY064517, KY064557. *P. heteractis* (Mett. ex Kuhn) Ching, India, Sikkim, Gangtok, M. Ohkubo (TNS 1184187), KY064507, KY064547, KY064570. *P. kinabaluensis* Hovenkamp, Malaysia, A. Sugawara *et al*. (TNS 764810), KY064518, KY064546, KY064569. *P. lanceolata* (L.) Farw., Vanuatu, G. Rouhan 637 (P 03945), KY064526, KY064545, KY064568. Kreier, H.P. & Schneider (2006), DQ164467. *P. linearifolia* (Hook.) Ching, Japan (cultivated in Tsukuba Botanical Garden), M. Nakata (TNS 763877), KY064516, KY064543, KY064566. *P. lingua* (Thunb.) Farw., Japan, Yakushima, S. Tagane & K. Fuse TF076 (TNS 763117), KY064522, KY064544, KY064567. *P. longifolia* (Burm. f.) C.V. Morton, Vanuatu, Efate, Iririki, S. Matsumoto (TNS 1184188), KY064525, KY064541. Kreier & Schneider (2006), DQ164501. *P. mannii* (Giesenh.) Ching, Kim *et al*. (2013), JX103715, JX103757, JX103673. *P. niphoboloides* (Baker) M.G. Price, Janssen *et al*. (2007), DQ642168, DQ642207. *P. nuda* (Giesenh.) Ching, Li,C. (unpublished), DQ078623, DQ078634. *P. nummularifolia* (Sw.) Ching, Thailand, R. Minagawa (TNS 768870), KY064519, KY064540. *P. pannosa* (Mett. ex Kuhn) Ching, S. Fujimoto (TNS 01184186), KY064529, KY064556, KY064577. *P. piloselloides* (L.) M.G. Price, Malaysia, M. Ohkubo (TNS 1184189), KY064514, KY064539, KY064565. *P. polydactyla* (Hance) Ching, Taiwan, A. Ebihara *et al*. (TNS 776622), KY064512, KY064537, KY064563. *P. porosa* (C. Presl) Hovenkamp, China, Yunnan, Simao, M. Ohkubo (TNS 1189777), KY064511, KY064536, KY064562. *P. rasamalae* (Racib.) K.H. Shing, Indonesia, Sumatra, M. Ohkubo (TNS 1184185), KY064510, KY064535, KY064561. *P. rupestris* (R.Br.) Ching, Schneider *et al*. (2004), AY362558, AY362623. *P. samarensis* (C. Presl) Ching, Janssen *et al*. (2007), DQ642170, DQ642209. *P. schimperiana* (Kuhn) Alston, Tanzania, Janssen & Rouhan 2624 (P 02766), KY064530, KY064542. *P. serpens* (G. Forst.) Ching, Schuettpelz & Pryer (2007), EF463260, EF463512. Kreier & Schneider (2006), DQ164503. *P. sheareri* (Baker) Ching, Taiwan, A. Ebihara *et al*. (TNS 776553), KY064509, KY064534, KY064560. *P. stigmosa* (Sw.) Ching, Indonesia, Sumatra, K. Kokubo (TNS 1184184), KY064506, KY064532. *P. subfurfuracea* (Hook.) Ching, cultivated plant, M. Ohkubo (TNS 1184177), KY064508, KY064533, KY064559.

Appendix 2. References for primers used in present investigation of *Pyrrosia*.

Primer	Reference
ES*rbcL*1F	Schuettpelz & Pryer 2007
ES*rbcL*645F	Schuettpelz & Pryer 2007
ES*rbcL*663R	Schuettpelz & Pryer 2007
ES*rbcL*1361R	Schuettpelz & Pryer 2007
*rbcL*336F	Pryer *et al*. 2004
*rbcL*888R	Pryer *et al*. 2004
*rps*4F1	Hennequin *et al*. 2003
trnS	Souza-Chies *et al*. 1997
*atpB*1163F	Wolf 1997
*atpB*1233R	Pryer *et al*. 2004
*atpB*1592R	Wolf 1997
*atpB*493F	Pryer *et al*. 2004
*atpB*672F	Wolf 1997
*atpB*910R	Pryer *et al*. 2004

APPENDIX 3. Number of sites, variable sites, parsimony-informative sites and percentage of missing data included in phylogenetic analysis for each marker investigated.

	rbcL	*rps4*	*atpB*	**Total**
No of sites (bp)	1174	622	358	2154
Variable sites (bp)	172	242	51	465
Pars-info sites (bp)	121	149	31	301
Missing data (%)	1	22	7	3.7

APPENDIX 4. Phylogenetic tree of *Pyrrosia* inferred by comparing sequences of DNA for the *rbcL* and *atpB* plastid genes and for the *rps4-trnS* intergenic spacer. Taxa with missing data are excluded. Values next to branches indicate, respectively, Bayesian inference posterior probability, ML probability and MP probability. Overall topology for taxa investigated is similar to the tree (Fig. 1) containing missing data.